MAPPING QUANTITATIVE TRAIT LOCI TO UNDERSTAND SEED SIZE VARIATION IN *CAMELINA SATIVA*

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Plant Science

MONTANA STATE UNIVERSITY
Bozeman, Montana

January 2019

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ABBREVIATIONS

RIL:	Recombinant inbred line
SPP:	Seeds per pod
TSW:	Thousand seed weight
GBS:	Genotyping-by-sequencing
SNP:	Single nucleotide polymorphism

ABSTRACT

Camelina sativa (L.) Crantz is an emerging *Brassica* oilseed crop. Camelina oil is high in polyunsaturated C18-fatty acids and its uses range from bio-fuels and bio-lubricants to an animal feed additive and cooking oils. A major breeding objective for camelina is to develop varieties with increased seed size. Understanding the genetics behind seed size variation would help breeders develop varieties that are more robust, easier to plant and harvest, better for oil processing, and could increase oil yield. For this study, a genetic linkage map was created and quantitative traits loci (QTL) were identified for eight agronomic traits using a bi-parental recombinant inbred population created between the two *Camelina* varieties: "Suneson," which has an average seed area of 1.35 mm^2, and "Pryzeth" with an average seed area of 2.24 mm^2. Field trials were conducted in 2017 and 2018 in both dryland and irrigated treatments in Bozeman, Montana. Significant QTL were discovered for seed size and other agronomic traits measured, including flowering time, pod size, seed weight, and oil content. The results of this study could lead to marker-assisted breeding for varieties better adapted to modern agriculture.

CHAPTER ONE

INTRODUCTION

Camelina sativa: Past, Current, and Future Production

Camelina sativa L. Crantz is a highly adaptable and productive member of the *Brassicaceae* family. Camelina has been known to humans in Northern Europe for thousands of years, with camelina seeds being found in cave deposits from the Neolithic age in Austria (Zinger, 1909). The dawn of agriculture saw several camelina species migrate into agricultural fields. These wild camelina species soon began to adapt to cultivation, especially in flax (*Linum usitatissimum*) fields. Camelina most closely resembles a "seed mimic" to flax, and in particular the subspecies *Camelina sativa* subsp. Linicola for its high degree of mimicry to cultivated flax (Barrett, 1983). This subspecies has a more slender and less branched stem than *Camelina sativa*, allowing it to compete more readily with flax, and it has bigger pods and seeds. Pods of subsp. Linicola play a major role in the mimicry, with the pods of subsp. Linicola not shattering as easily as *Camelina sativa* when ripe, allowing them to be harvested with the flax seed but not disperse naturally (Vavilov, Vavylov, & Dorofeev, 1992). This subspecies most likely arose due to the man-made selection pressure of the practices of threshing and winnowing flax seeds. Interestingly, the regions where small seeded varieties of flax seed were cultivated are where the large seeded *Camelina sativa* subsp. Linicola was found, and conversely, where the largest varieties of flax were cultivated, small seeded varieties of *Camelina sativa* were found (Sinskaia & Beztuzheva, 1930). This could be in direct

conflict with the idea of early cultivar selection based on seed size. However, this makes sense if seed separation (and in turn seed selection) is done through winnowing. Winnowing is the process in which air flow, either wind or generated by a machine, separates seeds from chaff. A larger seed with a greater surface area to weight ratio will be blown farther than smaller seeds. Flax seeds are much flatter and have a higher surface area than camelina seeds. Because of this, a small, rounded camelina seed will be blown a similar distance to large flax seed and vice versa. Because camelina mimics flax, some production areas of Northern Europe grew both flax and camelina side-by-side in the same field for combined oil production. Through either as a crop mimic or intentional domestication early producers soon realized that camelina seed produces a fine quality oil. However, unlike in other cases where weedy associate crops would take the place of basic crops, such as winter rye taking the place of winter wheat, camelina rarely replaced flax in monocrop production.

Camelina's center of origin is in Northern Europe, and in North America camelina production is being targeted for its Northern latitude, including the western prairie provinces of Canada, the North and Central Plains, and the Pacific Northwest. But like other *Brassica* oilseeds, camelina is highly adaptable to many environments. *Brassica* oilseeds are one of the few edible oil sources that can be successfully produced in the extremes of the temperate regions (Downey, 1983) and grown in the Southwest through Arizona, Southeast through Louisiana, and North to Pennsylvania (Berti et al., 2016). Camelina fell out of favor in Northern Europe, during the 1940s when superior developed crops such as wheat and rapeseed became more productive in those areas, and production of camelina remained low until a recent resurgence due to interest in its oil.

The main reason for increased interest in camelina oil is because of its remarkable ability to be used as a hydroprocessed renewable jet fuel (HRJ). Currently, it is being used in 50/50 blends with conventional jet fuels, and is a "drop in" alternative to petroleum based fuels (Berti, Gesch, Eynck, Anderson, & Cermak, 2016). Therefore, engine components such as fuel lines and pumps do not need to be changed in order to use camelina fuel in the engine. The US military, including the Air Force and Navy, and several commercial companies including KLM Royal Dutch and Japan Airlines have tested camelina fuel. The particle number and mass emissions from the exhaust of the fuel blend were compared to conventional jet fuel in flight for the first time by R. H. Moore et al. (2017). The authors found that compared to conventional jet fuels, partial number and mass emissions were reduced by 50 to 70 percent directly behind the aircraft.

To have a viable commercial oilseed, a market for the left-over meal from the oil pressing process must exist. For camelina, growers can use the meal as a feed additive for livestock and aquaculture. The seed meal is high in α - linolenic acid (18:3n-3) and can benefit livestock such as chickens (Cherian, Campbell, & Parker, 2009), pigs (Kim, Koo, & Nyachoti, 2017; Ponnampalam et al., 2019) and cattle (Brandao et al., 2018; Halmemies-Beauchet-Filleau et al., 2018) when camelina meal is included in their diets. One particularly promising market is aquaculture, and in particular salmon production. Currently, farmed salmon are fed feed combinations that contain fish oil harvested from smaller fish. The price of this oil is a major obstacle to the growth of the salmon aquaculture industry (Tacon & Metian, 2008). Because of its unique oil profile, camelina

could potentially replace fish oil in feed (Betancor et al., 2018; Hixson, Parrish, & Anderson, 2014).

Even with the interest in the oil, camelina production in Montana has been dropping. Production was at its highest in 2007 with 22,500 acres planted in Montana. This number has drastically declined in recent years with only 700 acres being reportedly planted in 2016. Primarily due to an historic low price of crude oil. Until now, most camelina in the US is contracted under through federal and private contracts for biofuel, and with low oil prices these contracts are not widely available. Also, for the most part no open market camelina oil exists to sell seeds under contract. Currently, most camelina is sold is direct market sales at places like farmers markets where it is marketed as a cooking oil.

Lack of substantial present production has created a gap in knowledge when producing camelina with modern agricultural techniques. Production practices still need to be standardized to include proper plant density, row spacing, and fertilizer makeup and amount. In addition, camelina has not been intensively bred and many traits need to be improved. Several breeding objectives have been laid out by Berti et al. (2016), including early-maturing strains, an increase in seed oil and meal protein content, the resistance to biotic stresses such as disease and insect pests, resistance to broadleaf herbicides, and an increase in seed size.

The development of early-maturing strains would be useful because of the intriguing possibility of double-cropping camelina with higher grossing crops. Camelina is naturally a great candidate for this type of production because of its short growing season (~ 92 days), and maturity can even be hastened further via swathing or via

chemical desiccants. When double-cropping was studied in a camelina-soybeans cropping system, no yield loss was seen in the camelina crop, even with the use of either swathing or desiccation, and soybeans yields were 58 to 83% of what the mono-cropped control produced (Gesch & Archer, 2013). The soybean in the camelina-soybean cropping system had combined oil yields as much as 50% higher compared to a soybean-soybean cropping system. When double-cropped in a camelina-sunflower cropping system, the late-sowed sunflower crop can yield up to 82% of a mon-cropped control (Gesch, Archer, & Berti, 2014). Double-cropping with winter camelina varieties could potentially see an increase in camelina yield without a large yield penalty for the second crop being grown because camelina could be harvested earlier. Winter cultivars of camelina rival the winter hardiness of winter rye (*Secale cereale* L.) and far surpass the winter hardiness of winter annual oilseed rape (Schillinger, Wysocki, Chastain, Guy, & Karow, 2012). Also, due to the exposure to cold temperatures, winter cultivars could produce a higher percentage of unsaturated fatty-acids than spring camelina cultivars (Raziei, Kahrizi, & Rostami-Ahmadvandi, 2018). Winter cultivars require vernalization, but vernalization has not been studied extensively in the field. Séguin-Swartz, Nettleton, Sauder, Warwick, and Gugel (2013) reported successful vernalization in a vernalization chamber of 4°C with a 12-hour photoperiod for 6 weeks.

Another breeding goal is partial/total resistance to broad-leaf herbicides that are commonly used in small grain cereals cropping systems, which include wheat and barley. This has been successful in the case of acetolactate synthase (ALS) inhibitors (Hanson, Park, Mallory-Smith, & Thill, 2004; Walsh, Babiker, Burke, & Hulbert, 2011). Allows

camelina to be grown in rotation with cereals where current camelina production is focused.

One breeding goal that could potentially lead to much higher yields and better agronomic performance is to take advantage of heterosis using hybrid seed. Heterosis creates stronger, more vigorous plants in *Arabidopsis* (Stokes, Morgan, O'Neill, & Bancroft, 2007) and has become a common breeding practice in canola (*Brassica napus).* The possible advantages of heterosis in camelina were tested through pairwise crosses of 13 individual plants by Zelt and Schoen (2016) with some progeny displaying best-parent heterosis for traits associated with seed yield. Camelina, like canola, is a self-pollinating crop with perfect flowers, and a low outcrossing rate of only 0.01 to 0.28% (Walsh et al., 2011). This can make creating a significant number of crosses highly difficult, and unique approaches to developing hybridization need to be developed. One way is to create cytoplasmic male sterility (CMS) through the use of gametocidic herbicides. These herbicides disrupt gamete development, resulting in sterile pollen. Spraying camelina plants with trace amounts (0.2 and 0.4 mg/L) of the sulfonylurea herbicide tribenuron-methyl is sufficient in inducing 80 to 100% male sterility, and 87% and 54% manually pollinated seed-set rate respectively (Yu, Dong, Hu, & Xu, 2017). This was also seen with low toxicity to pistil function. The use of gametocides could be a cheap and effective method of making the high number of crosses required to exploit heterosis in camelina.

We can also look at the canola ideotype to see where future breeding efforts in camelina should be targeted. Early canola varieties were typically tall plants with thick stems and few branches, which started from a high position on the stem (Al-barzinjy,

Stølen, & Christiansen, 2003). New cultivars are generally shorter, bearing more primary branches that develop earlier and start from a low position on the stem. Often, these newer cultivars have smaller leaves so lower branches receive enough light to make them productive. Interestingly, larger seeded plants show a tendency to produce leaves with greater leaf area (Mendham, Shipway, & Scott, 1981). Potentially having a negative effect on yield. Branching structure is a good predictor of yield, and unlike other annual crops such as cereal crops, which are adapted to producing one tiller per plant, *Brassica* oilseeds need to take advantage of branching. Even in low density plantings, *B. napus* plants have been shown to fill in and compensate with higher branch and pods per plant numbers (Mendham et al., 1981). This has also been found to be true in camelina. McVay and Khan (2011) found that a stand reduction through thinning of plants—at both the rosette and bolting growth stages of up to 50%—had no significant effect on grain yield over a 2-year period.

Genetically modified camelina could play a major role in future cultivation. Over the past decade, researchers have genetically altering camelina because of its close genetic relationship to the diploid model species *Arabidopsis*, and its ability to be easily genetically transformed (Malik et al., 2018). Lu and Kang (2008) demonstrated a method to easily and effectively transform camelina using *Agrobacterium*. With the emergence of CRISPR/Cas technology, there has been an increase in studies using camelina as a model hexaploid to create novel mutations. Aznar-Moreno and Durrett (2017) were able to use carefully designed guide RNAs to demonstrate the ability of the CRISPR/Cas genome editing system to introduce mutations in all three *CsDGAT1* and *CsPDAT1* genes homologous genes important in oil synthesis. The CRISPR/Cas system was also

demonstrated to work on multiple copies of *FAD2*, a gene important in fatty acid desaturation (Jiang et al., 2017*)*. Ozseyhan, Kang, Mu, and Lu (2018) demonstrated the system on the reduction of three copies of *FAE1*, an important gene to very-long chain fatty acids synthesis that needs to be reduced to make camelina more suitable for food/feed use. The introduction of modified camelina into breeding programs could help breeding objectives be realized faster than simply using traditional breeding efforts.

CHAPTER TWO

LITERATURE REVIEW

Evolution of Seed Size in *Brassicaceae*

Most of the plants consumed today have seeds far larger than their wild relatives, and for many crops, seed size is an observable change that enables us to determine when a plant species may have been domesticated (Purugganan & Fuller, 2009). The selection for larger seeds may have occurred directly post-harvest, or indirectly through selection of plants with larger seedlings in open environments during the growing season. Larger seeds are better adapted to be sown at greater depths during tillage practices that accompanied early agricultural practices.

In general, *Brassica* oilseeds are thought to be one of the earliest crops to be domesticated and have a large variety of seed size and seed weight. In fact, an initial way to tell different members of the genus apart was with the size of their seed (Musil, 1948). For example, white mustard genus (*B. alba*) has large seed compared to something like wild turnip (*B. tournefortii*), which has a much smaller seed. Currently, all *Brassica* oilseeds in production have larger seeds than camelina, which has a seed weight of 1g/1000 seeds. Some examples are summer field mustard (*B. campestris)* with a seed weight of 2-3 g/1000 seeds and winter rapeseed *(B. napus)*, which has a seed weight of 4.5-5.5 g/1000 seeds, and in general the winter forms of *Brassica* oilseeds produce larger seeds than those found in the summer types (Downey, 1983). Camelina seed size is small

in cultivated Brassica crops but is average for the plant Family *Brassicaceae* (Moles et al., 2005).

Genes that influence seed size in *Brassicaceae* seem to be fairly consistent amongst members of the family. When comparing *Brassica* species genomes to *Arabidopsis* genome, G. Cai et al. (2012) found 398 homologous genetic loci between the genomes for seed weight.

Seed Size and *Brassica* Oilseed Production

Seed size is not only a major yield component, but it is also important to much of growth physiology. Especially for early plant growth, including both germination and seedling establishment. In general, large seeds have better field performance than small seeds (Ambika, Manonmani, & Somasundaram, 2014). A classic series of studies on the effects of seed size on germination were been conducted in *Trifolium subterraneum* (Black, 1956, 1957, 1958). In these studies, three different sizes of a single variety were sown at three different depths (1.3, 3.1, and 5 cm), and when these three different sized seeds were sown at the same time, the photosynthetic area of the cotyledons decreased with increased depth and decreased seed size. Basically, the cotyledon weight after emergence paralleled the size of the seed sown. These studies also showed that the photosynthetic area of the cotyledons was critically important in the growth of the emerged seedling.

The role of seed size on production in camelina has not been extensively studied. However, we can look at other *Brassica* oilseeds and *Arabidopsis* for insight.

First, in *Arabidopsis*, several studies have been conducted on seed morphology. When *Arabidopsis* is grown in sterile petri dishes under lab conditions, large-seeded varieties have seedlings with a longer survival rate (Krannitz, Aarssen, & Dow, 1991). Seed size plays a role in early plant growth. In the growth chamber environment young plants of *Arabidopsis* that develop from larger seeds also have higher plant biomass. However, the biomass of smaller seeded varieties have been shown to catch up to the larger seeded varieties as the plants mature (El-Lithy, Clerkx, Ruys, Koornneef, & Vreugdenhil, 2004),

Brassica napus has a wide range of seed sizes and many studies have been conducted on the topic. Larger *Brassica napus* seeds have better emergence and establishment (Harker et al., 2014), and young *Brassica napus* plants from larger seeds have the potential to have higher biomass (Elliott, Franke, & Rakow, 2008; Harker et al., 2016). In turn, plants with better establishment and higher biomass may compete better with weeds. Finch-Savage, Clay, Lynn, and Morris (2010) showed in *Brassica oleracea* that an increased seed weight has a positive influence on the ability of hypocotyls to grow through hard soil. The authors did not find an obvious link between seed size and germination characteristics but acknowledged that, in general, increased seed size benefited seedling establishment.

A larger seed can also benefit agronomic traits during the growing season such as important growth stages including flowering and maturity. A larger seed may decrease both days to flowering and days to maturity (Harker et al., 2016). In *Brassica napus*, larger seeded varieties decreases the time from seedling to the start and end of flowering by one day and the time to maturity by two days compared to small seeded varieties.

Producers could also benefit from larger seeded varieties. This is because in general larger seeds are much easier to handle than small seeds. The biggest benefit would come during harvest. Camelina can be harvested using a standard combine used for canola harvest. However, current small seeded varieties tend to be difficult to harvest. The smaller seeds are often left in the field. This can happen either at the front of the combine as the plants are being cut, or at the back of the combine where plant material is being left on the field. A study conducted by Hunter and Roth (2010) at Pennsylvania State University saw that as much as three pounds of seeds per acre can be lost back to the field during combine harvesting. Causing economic costs in several ways. First, loosing seed in the field results in a yield loss over large production acres, leaving money in the field. Second, camelina seeds lost in the field could become volunteers during other crop rotations. Volunteers create more work for the producer and could also create yield penalties for other crop rotations. Producers that are trying to mitigate loss will combine slower and on the dirtier side, meaning that they will harvest and store more of the plant material with the seed as it is being harvested. This creates further steps of seed cleaning before the seeds can be pressed for oil. Another advantage to producers could be higher quality oil.

In *Brassica napus* a larger seeded plant has been shown to have reduced amounts of harvested green seed over smaller seeded plants (Harker et al., 2016). In camelina this reduction of underdeveloped green seed could lead to higher quality oil and more profit to the producer.

Seed Development in *Brassicaceae*

Consistent with other higher plants, seeds of the *Brassicaceae* family consist of three major components: the embryo, endosperm, and seed coat. The beginning of seed development is marked by the rapid growth of the endosperm and the embryo (the endosperm grows much more rapidly than the embryo) until seed maturation; which is followed by desiccation (Sundaresan, 2005). The ovule develops from the maternal tissues. Rapid increase in seed volume occurs in concordance with the rapid growth of the endosperm. The embryo is largely dominated by an inner and a larger outer cotyledon. These are arranged in a conduplicate fashion. In *Brassica napus* few differences in the seed size were found between a large-seed line and a small-seed line one to two weeks after flowering (Geng et al., 2018). However, from three to six weeks after flowering, seed weight of the large-seed line was higher than the small-seed line. In *Brassicas*, the endosperm is eventually consumed, and is replaced by the growing embryo with the cotyledons replacing the endosperm as a storage organ. Brassica oilseeds are predominantly embryo tissue as opposed to cereal grains which are largely endosperm (Downey, 1983).

The size of the seed is the result of three different growth programs: diploid embryo (♂♀), triploid endosperm (♂♀♀), and the diploid maternal ovule (♀♀). Traits that affect seed weight could be ranked in the following order: seed coat cell number, cotyledon cell number, seed coat cell size, and cotyledon cell size (Li et al., 2015). The maternal effects on seed size have also been seen in *Arabidopsis* in reciprocal crosses where progeny from larger seeded female parents had 41% heavier seeds (Alonso-

Blanco, Blankestijn-de Vries, Hanhart, & Koornneef, 1999). Similarly, De Jong, Hermans, and Der Veen-van Wijk (2011) used 25 'male' accessions to pollinate the 'female' accessions Col and l*er*. The authors let both Col and l*er* self-pollinate, and they outcrossed both to the 25 'male' accessions. Finding no correlation between seed size of the paternal parent and the mass of the seeds the paternal parent sired. Bigger paternal seeds did not sire bigger seeds. In B*rassica napus*, Li et al. (2015) also found that seed weight is mainly controlled through the maternal genotype. They showed that in crosses involving small-seeded lines pollinated by large-seeded lines the maternal effect had a mean genetic value of 0.90 (embryo-cytoplasm-maternal model for genetic variance between 0 and 1). Li et al. (2018) crossed two inbred lines that were large and small seeded. The calculated maternal effects of the F_1 hybrids and the inbred parents was 0.97 and 0.88, respectively, a small cytoplasmic effect on seed weight was found.

Genome of *Camelina sativa*

The genome of *Camelina sativa* is a hexaploid with a size of 785 Mb contained on 20 chromosomes and a predicted 89,418 non-redundant genes, and was sequenced by Kagale et al. (2014), The *Camelina sativa* has approximately three times as many total genes as *Arabidopsis*, a diploid. Interestingly, the gene number is similar to the number predicted for bread wheat which has a genome that is almost 22 times the size of *C. sativa.* The hexiploid genome of *C. sativa* was likely formed through inter-specific hybridization between lower chromosome ancestors, most likely diploids, because of strict maintenance of homologous recombination between highly syntenic sub-genomes. This is similar to *Brassica napus* (AACC, 2n = 38), which originated from a

allopolyploid polyploidization event between the two diploid ancestors, *Brassica rapa* (AA genome, 2n = 20) and *Brassica oleracea* (CC genome, 2n = 18) (Shi et al., 2015). Also similar to *Brassica napus*, the genome of *C. sativa* has a strikingly high level of conservation between two *Arabidopsis* species (*A. lyrate* and *A. thaliana*), with each chromosome of both Arabidopsis species represented in three independent chromosomes in the *Camelina sativa* genome. This provides robust evidence for a whole-genome triplication event. Interesting, *Camelina sativa* behaves like a diploid with normal disomic inheritance, even though it has nearly identical sub-genomes.

Brock, Dönmez, Beilstein, and Olsen (2018) suggest that the wild small-seeded *Camelina microcarpa* is the wild relative of *Camelina sativa*. The two species have the same number of chromosomes (n = 20) and similar genome sizes. *Camelina sativa* is rarely found outside of fields. Brock et al. (2018) collected 54 accessions in camelina's center of origin of Northern Europe and found only one outside of an agricultural context which seemed like an accidental introduction. Also, interestingly, there are no wild summer annuals of this genus. This suggests that the summer annual habit of *Camelina sativa* arose as a result of selection by early man (Stebbins, 1950).

Linkage Map Construction and QTL Discovery

A logical beginning step to understand genetics for a highly quantitative trait is to locate areas of the genome that influence the trait. This can be done through a quantitative trait loci (QTL) mapping study (Acquaah, 2012). Where regions of the genome that effect quantitative traits of interest associate with molecular markers. For these studies a genetic linkage map needs to be created or used from a previous study. A

linkage map shows the position of known genetic markers relative to one another on linkage groups. These linkage groups are constructed by calculating recombination frequencies between genetic markers. Several different techniques have been used to create molecular marker for linkage maps of the *Camelina sativa* genome. So far, most of the linkage maps published *C. sativa* have been from a recombinant inbred line (RIL) population of the two *Camelina sativa* varieties 'Lindo' and 'Licalla'. The first linkage map was created by Gehringer, Friedt, Lühs, and Snowdon (2006) and used by Enjalbert (2012). It was constructed with 157 AFLP and 3 SSR markers forming 20 linkage groups. Kagale et al. (2014) mapped the same cross with 3,575 SNP markers to form 20 linkage groups that were then located onto the 20 chromosomes that make up the *Camelina sativa* genome. A more recent linkage map was constructed using next generation sequencing techniques and single nucleotide polymorphism (SNP) calling to map 533 SNP markers onto 20 linkage groups (Singh et al., 2015). Ayala Diaz (2014) used a mapping population of F_2 lines created from two accessions from Denmark to create a linkage map containing 767 SNP markers.

The previous studies for QTL analysis in camelina used composite interval mapping for QTL discovery (Ayala Diaz, 2014; Enjalbert, 2012; Gehringer et al., 2006). Composite interval mapping has advantages over other mapping techniques because it combines simple interval mapping with multiple regression statistical analysis allowing other markers effects on the same chromosome to be controlled (Acquaah, 2012). This allows composite interval mapping to accommodate for missing genotypic data.

QTL Studies Related to Seed Size

As would be expected, a number of QTL studies have mapped seed oil content in *Arabidopsis* and *Brassica* oilseeds. Like many other important agronomic traits, seed oil content is controlled by a complex network of genes and understanding of that complex genetic control has benefited from mapping. Seed size is equally complex and has also benefited from mapping. The genetics behind seed size was first laid out by Harper, Lovell, and Moore (1970). However, the genetic interactions and networks controlling seed size are still poorly understood. In camelina three studies have located QTL for agronomic traits (Ayala Diaz, 2014; Enjalbert, 2012; Gehringer et al., 2006). These studies localized QTL for seed yield, oil content, 1000-seed mass, plant height, and flowering. Significant QTL were located for all traits, and several of the QTL located were confirmed between studies.

Multiple QTL studies have been published for seed size in *Arabidopsis thaliana* (Alonso-Blanco et al., 1999; El-Lithy et al., 2004; C. R. Moore, Gronwall, Miller, & Spalding, 2013; Rowan, Robert, Samantha, & Richard, 2011; Van Daele et al., 2012). In these studies, RIL populations derived from bi-parental crosses were used. Alonso-Blanco et al. (1999), using a RIL population derived from the accessions Cvi and Ler, found 11 QTL for seed weight and/or seed length. Each locus explained between 2.5% and 12%, and combined they explained 56.2% of seed length and 71.5% of seed width variation. These QTL were located in the short arm of chromosome 1, middle and long arem of chromosome 5, and the long arm chromosome 4. This same population was used by Van Daele et al. (2012) and C. R. Moore et al. (2013) who used seed area

measurement instead of seed weight. Combined, they located a total of 8 QTL for seed size. C. R. Moore et al. (2013) confirmed the location of QTL on chromosome 1. Rowan et al. (2011) used two different bi-parental populations and were able to locate 9 QTL for seed size and confirmed locations of QTL on chromosome 4. El-Lithy et al. (2004) used a bi-parental population derived from Ler and Sha and revealed one seed weight QTL on chromosome 5.

Over 130 QTL have been detected for thousand seed weight (TSW) in *Brassica napus* (Fan et al., 2010; Fu et al., 2015; Li, Shi, Wang, Liu, & Wang, 2014; Quijada, Udall, Lambert, & Osborn, 2006; Radoev, Becker, & Ecke, 2008; Shi et al., 2009; L. Sun et al., 2018; Yang et al., 2012; Zhang et al., 2011; Zhao et al., 2016). The majority of the QTL detected explain no more than 10% of phenotypic variance. Only a few show higher phenotypic variance and have been validated. Multiple studies have identified the same QTL on chromosome A7 (A for the A genome of *Brassica napus)* , describing between 5.63% and 20.8% (D. Cai et al., 2014; Fan et al., 2010; Radoev et al., 2008; Shi et al., 2009). Another significant QTL A9 explained as much as 28.2% of the variation in seed weight (Fu et al., 2015; Shi et al., 2009; Yang et al., 2012). Interestingly, this QTL on A9 co-locate with silique length, and can explain as much as 53.4% of the variation in silique (pod) length (Shi et al., 2009). The co-localization of QTL for seed weight and silique length has been shown in multiple studies (Fan et al., 2010; Li et al., 2014; Yang et al., 2012; Zhang et al., 2011).

CHAPTER THREE

MATERIALS AND METHODS

Bi–Parental RIL Population

The accession used as the female parent of the bi-parental RIL population, 'Pryzeth' (Pry), is a subspecies of *Camelina sativa*, known as *Camelina sativa* subsp. Linicola. This subspecies differs phenotypically from *Camelina sativa* in that it has more slender stems, branch stems with longer internodes, more glabrous leaves (free from trichomes), inflorescences with fewer flowers, and especially larger pods and seeds (Stebbins, 1950). These are key morphological characteristics that can be used to distinguish camelina species (Brock et al., 2018). This subspecies is fully fertile with *Camelina sativa* and segregates in crosses with *Camelina sativa* to produce a great array of intermediate phenotypes.

The accession used as the male pollinator parent of the bi-parental RIL population,'Suneson' (Sun) is a true member of *Camelina sativa* ssp. *sativa* and widely used as a genotype/phenotype in experimentation.

These two accessions were crossed producing 190 progeny that were advanced to the F_5 generation using single seed decent to create a RIL population. F_5s were tested in the 2017 field season. Single plants were selected from 2017 plots to advance the population to the F_6 generation for use during the 2018 field season.

Field Trials

Field trials were performed at the Arthur H. Post Research Farm in Bozeman, Montana. The 190 RILs were planted in an augmented randomized complete block design with one-way blocking that was first described by Federer (1956). The RILs were planted in both dryland and irrigated treatments with 10 blocks per treatment in 2017 and 5 per treatment in 2018. Each RIL was planted in a plot consisting of two, ten foot rows, with one foot spacing between rows. Four check varieties were used, including both the parents of the population, and two commonly grown varieties of *Camelina sativa* 'Blaine Creek' and 'Calena'. In the Fall of 2017 (10/24/2017), the pre-emergent herbicide Treflan™ (Dow AgroSciences) was applied to the field area at a rate of 1.5 pints/acre. Immediately after spraying, the herbicide was worked into the soil at a depth of 4".

The plots in both years were mechanically planted using a six-row planter from the Hege Company (Waldenburg, Germany). In 2017 plots were space planted at a rate of 1 lb/acre. 1lb/acre of seed equals ~9 seeds per square foot (McVay & Lamb, 2008). For the 2018 growing season a higher planting rate of 3 lb/acre was used. No additional fertilizer was added, and plots were maintained using standard agricultural practices.

Overhead sprinklers were used for additional water applied to the irrigated treated fields. In 2017, the irrigated field received five irrigation events, spaced evenly, during flowering and pod formation, totaling an additional 12.7 cm of precipitation. Irrigation was performed during the same life stages in 2018 with five irrigation events adding an additional 11.43 cm of precipitation. Weather data for the Author H. Post research farm

was collected from the NOAA National Centers for Environmental information Network:ID = GHCND:USC00241047.

In both 2017 and 2018, when plots reached maturity, subsampling was performed by harvesting main racemes from five individual plants in the middle of each plot. From these five racemes, 20 well developed pods were taken from the middle of each (at least 10 pods up from the bottom) to lessen variability between samples. Similar techniques were used by previous researchers (Li et al., 2018; Mahmood, Rahman, Stringam, Yeh, & Good, 2005). After subsampling was finished, full plots were harvested. In 2017, a single-row binder was used to cut and bind plots, and then bundles were left in the field for 2-3 days to dry. A Vogel Plot Thresher was then used to separate seeds from bundles. In 2018, plots were bulk harvested using a Wintersteiger plot combine.

Agronomic Trait Measurement

In total, 8 agronomic and seed quality traits were measured during the growing season and post-harvest period. These traits included days to flowering, days to maturity, plant height, seed size (area, width, and length), pod size (area, width and length), seeds per pod (SPP), oil (%), and thousand seed weight (TSW).

Days to flowering was measured when 50% of the plants in each plot showed their first flower. Days to maturity was recorded when 50% of the pods on 50% of the plants in each plot reached maturity and were brown with mature seed. Height was measured using a standard meter stick from the base of a single plant to the top of the main raceme and replicated three times per plot.

The traits of seed area (mm^2), seed length (mm), seed width (mm), pod area (mm^2), pod length (mm), and pod width (mm) were measured with scanned images. The images were captured on an Epson Perfection V600 Photo Scanner at 600 dpi (0.024 mm/pixel) with the supplied software, without image enhancement and saved as TIFF (tagged image file format) files. The samples were processed in several steps. first, the 20 pods from main racemes were spread out evenly on the glass of the scanner and scanned. Then, the pods were threshed, and seeds were separated from the pods and cleaned. All the seeds (n = at least 148 and as many as 421 seeds) from the 20 pods were then evenly spaced on the glass of the scanner and scanned. This process was repeated for all samples. SPP was calculated as, SPP = (total seeds from sample / 20). The scans were imported into the *SmartGrain* program for analysis, *SmartGrain* was created as software for high-throughput seed phenotyping (Tanabata, Shibaya, Hori, Ebana, & Yano, 2012). *SmartGrain* calculates seed/pod area as, AS = $P_i(x_i,y_i)$, a set of perimeter coordinates. Seed length and pod length were measured by detecting the maximum distance between two points on the perimeter, and seed/pod width was measured by detecting the longest segment that is perpendicular to the length segment. The resulting phenotyping analysis were extracted as a Microsoft® Excel document.

For each RIL, three replications of total oil (%) were measured using an Oxford Instruments MQC benchtop NMR analyzer. The ~250 mg samples used to measure total oil in the benchtop NMR analyzer were then used to measure TSW with the equation TSW = (sample weight (mg) / sample total seed #) x 1000. To get the sample total seed number a Seedburo COUNT-A-PAK Seed Counter was used.

Statistical Analysis

The augmented randomized complete block design requires checks to be replicated but does not require full replication. A way to get a better estimate of variance without full replication is to calculate BLUPs (best linear unbiased predictors) for each trait. BLUPS were calculated using a mixed linear model of:

$$\mu\{y|entryc, block, entry{:}new\} = \beta_0 + \beta_1 entryc + \beta_2(1|block) + \beta_3(1|entry{:}new)$$

where y was the trait of interest, entryc was a column in the dataset where the 190 RILs are in a single pool and the checks are treated separate, block is ether 10 for 2017 or 5 for 2018, entry was all lines including the 190 RILs and all 4 checks, new was a column in the dataset where a 0 is assigned for checks and a 1 for a RIL. In this model the 190 RIL are considered a random effect and checks are a fixed effect. To make this data easier to deal with, EBLUPs (empirical best linear unbiased predictor) for each trait were calculated. EBLUP = RIL overall mean for a single trait + BLUP.

Mean differences between Sun and Pry were calculated using two-sample T-Tests. Differences between years and treatments for the RIL population were calculated through individual trait ANOVAs from mix model regression equations that included year, treatment, and entries. Broad-sense heritability was calculated using the equation:

$$H^2 = \sigma^2_g/(\sigma^2_g + \sigma^2_{ge}/n + \sigma^2_e/nr)$$

where, σ^2_g was the genotypic variance, σ^2_{ge} was the interaction variance of the genotype and environment, σ^2_e was the error variance, n was the number of environments, and r was the number of replications.

All statistics, including those listed above, Person correlation coefficients, year to year means, standard errors, standard deviations, and data ranges were calculated in the statistics software R v1.1.383.

Imaging of Parental Lines Seed Development

Pods were collected from greenhouse-grown camelina plants at different developmental stages after anthesis/flowering (DAF). Seeds were removed from seed pods and imaged immediately using a dissecting microscope coupled with Nikon imaging system. To visualize and measure cell size and cell number of seed coat, epidermal layers were peeled off from 21 DAF embryo tissue of Sun and Pry and flattened by tweezers. Samples were placed on slides and observed under a Nikon microscope with bright field illumination.

GBS, SNP Calling, and Linkage Map Construction

DNA extraction of 190 F_5 RILs and the two parents was carried out using the FastPrep™ DNA Extraction and Purification Kit (MP Biomedicals). DNA concentrations were measured with the Quant-iT™ PicoGreen™ dsDNA Assay Kit (ThermoFisher Scientific, Waltham, MA, USA) and 1 µg of gDNA from each line was dissolved in 10 mM Tris-HCl pH 8.0 to a final volume of 50 µl. GBS was performed at LGC Genomics (Berlin, Germany) with MsII enzyme utilizing the Illumina NextSeq® 500. After read pre-processing, GBS clustering and alignments were done using Bowtie2 version 2.2.3. Variant discovery and genotyping of samples were performed with Freebayes v1.0.2-16.

The markers with significant segregation distortion ($P < 0.05$) were removed through chi-square tests combined with sequential Bonferroni correction. Markers with >15% missing rate and those that were monomorphic and distorted (differing significantly from the expected 1:1 segregation ratio) were eliminated from the analyses. A genetic linkage map was constructed using MapDisto 2.0 (Heffelfinger, Fragoso, & Lorieux, 2016). In MapDisto, the markers were grouped with a recombination fraction of 0.3 and logarithm of the odds ratio (LOD) score of 7 using the Kosambi mapping function. The Seriation algorithm was used for ordering the linkage groups. Linkage groups were assigned to chromosomes by BLAST using several SNP markers from each linkage group against the camelina genome database (http://camelinadb.ca/). Chromosomes were found to be divided into multiple linkage groups, so these linkage groups were combined and re-ordered. Rippling of marker order with a window size of five markers and checking for inversions were performed to improve the marker order and produce the shortest map of each chromosome.

Map calculations were performed using the Kosambi function and Maximum Likelihood Mapping of the program Qgene v4.4.0. Markers and individuals were excluded from the analysis if they exhibited more than 10 and 20% missing data, respectively. For individual chromosome maps of the two parental lines, initially a first map was calculated and then subjected to manual correction. Non-fitting markers were excluded, and the maps were recalculated until no uncertain marker positions remained. Removing non-fitting markers sufficed to reach a stable map. The resulting marker order of each map was compared with previous genetic maps of *Camelina*.

QTL Analysis

QTL Analysis was performed using the software Qgene v4.4.0 (Joehanes & Nelson, 2008), using 1803 SNP markers. Discovery was performed with composite interval mapping with cofactor analysis. Cofactor selection was done automatically through Qgene, using a forward step-wise regression model. Maximum number of co-factors was set to 3 times the number of traits in the model, F-to-add threshold is computed as P = 0.05 cutoff value on the inverse CDF (cumulative distribution function) of an F distribution with numerator df = 1 and denominator df = number of markers in the data set, and F-to-drop threshold is computed as F-to-add minus 0.1. The window size was 1cM. A QTL was considered significant when a permutation test, with 1000 random permutations, was performed and the LOD was higher than the permutation test's 0.05 alpha level. If the QTL was significant in more than one environment than the LOD, additive effect, and R^2x100 values from the mean environment were reported. QTL chromosome position was confirmed using the BLAST function provided by the *Camelina sativa* Genome Project (http://camelinadb.ca/).

CHAPTER FOUR

RESULTS

Phenotypic Summary of Traits Over Years and Treatments

In this study, seed size and pod size are characterized by the measurements of area, length, and width. Three measurements were used to more accurately locate seed-size QTL.

Seeds from the parental line Pry were, on average, have a 66% larger area and are 71% heavier than seeds from Sun (Table 1). The pods from Pry were also, on average, have a 80% larger area than Sun pods. Significant differences ($P < .001$) were measured between the parental means of all traits. The distribution of the 190 RILs for all phenotypic characteristics measured show a normal distribution (Figure 1), and for several agronomic traits there are members of the population that fall outside of the distribution of the two parents and show transgressive segregation. This is most evident with higher and lower seeds per pod (SPP), but also present with lower oil (%), both shorter and longer days to flowering and days to maturity, and some of the population have shorter height (cm) than either of the two parents.

The F_5 generation was planted in 2017 and F_6 in 2018, and the means and SE for each trait in each year are presented in Table 2. For each agronomic trait an ANOVA was calculated for year to year differences and treatment differences to find significant differences. Year to year significant differences ($P <.001$) were seen for all traits except oil (%), days to flowering (DTF), and days to maturity (DTM). Treatment significant

differences were seen for all traits except SPP and oil (%). All traits show a wide range of variability when averaged over both years and treatment. Table 2 also shows broad–sense heritability (H^2) of all agronomic traits, with many of the seed and pod traits showing high heritability. This is especially true for seed area, length, and width with H^2 values of 0.85, 0.88, and 0.79, respectively. Interestingly, for this population oil (%) has a relatively low H^2 value of 0.66.

A Pearson correlation coefficient matrix is presented in Table 4 for all agronomic traits over both years and treatments. Both seed length and width are highly positively correlated to seed area (r=0.92 and 0.88, respectively). Pod area and seed area show a weak positive correlation (r=0.39). Seed area has a negative correlation with SPP, and pod area has a positive correlation with SPP (r = -0.30 and 0.39, respectively). Seed width and length are highly correlated (r=0.65); however, this same correlation is not seen between pod width and length (r=0.31). Seed area and oil percentage are slightly negatively correlated (r=-0.24).

Figure 2 represents the trait complex of seed area, pod area, and SPP. The data in the figure represent all data sets from both years and treatments. The forward pane of the three-dimensional figure shows that as seed area is increased, the number of seeds per pod is decreased. The rest of the figure is the inclusion of pod area. SPP increases with increasing pod area. The greatest number is shown with large pods and small seeds, and the lowest with small pods and large seeds. The RIL population shows a great variation between these three traits.

The development of seeds from the parents of the bi-parental population is shown in Figure 3. Sun and Pry show similar sizes during morphogenesis, then the main

increase in seed size happens during the maturation stage of development, and Pry continues to be bigger all the way through seed desiccation. The photos below the development stages show a layer of cells that represent the mature seed size of Sun and Pry. It is evident from the photos that Pry has a larger mature seed in part because of an increase in cell expansion.

Weather and Treatment Summary

Dates of planting were very close between the 2017 and 2018 field seasons with only one day apart (May 5th, 2017 and May 4th 2018). However, on average, after May, 2017 was a warmer year than 2018 with much warmer temperatures in June and July (average difference of 2°C in June and 3.1°C in July), which coincided with flowering and pod formation stages that are critical periods for seed development (table 3). Overall 2018 was a wetter year than 2017 with 6.35 additional centimeters of precipitation over the growing season. The application of irrigation occurred over the same period in both years, with 2017 receiving an addition 1.27 cm.

Linkage Map and QTL Summary

The Pry/Sun population was genotyped by sequencing to generate SNP markers. Starting with 3225 markers, SNPs were discarded that contained >15% missing data. This reduced the number of markers to 2203. Markers were again eliminated if a chi-squared test resulted in a significance value of < 0.05. This reduced the markers 2003 SNPs that were mapped onto twenty linkage groups using a recombination fraction of

0.3, and a LOD minimum of 7. These linkage groups were used for subsequent QTL analyses. Markers on each linkage group were scrutinized with BLAST provided by the camelina reference genome and each linkage group was successfully assigned to the corresponding chromosome. Table 5 shows an overall summary of the genetic linkage map constructed using MapDisto 2.0.

QTL were identified for explaining a portion of the variation of all seven phenotypic traits evaluated. Table 6 and Table 7 list significant QTL marker names, chromosomes, and peak positions closest to the identified QTL reported, as well as associated LOD scores, R^2 values, additive effect values from the Suneson allele, and environments present. If a QTL was present in more than one environment, that included the mean environment, than the mean LOD, R^2 and additive effect values are reported from the mean environment. If the QTL was only present in one environment, then the values from that environment are reported.

A total of 27 significant QTL were detected on 15 of the 20 *Camelina sativa* chromosomes, and 15 of these QTL located were found to be significant in multiple environments. Six of these QTL co-located for multiple traits (Table 7). These co-locations defined as overlapping of peak LOD scores for two or more traits. Three QTL located for DTF and DTM, and all three co-located together. These QTL explain 20.1% of total phenotypic variance for DTF, and 21.9% for DTM. One of these QTL, *qMTDEV-7*, also co-located for height. In addition to that QTL, three more significant QTL were located for height explaining between 6.9% and 10.6%, and a total of 34.4% of the phenotypic variance. The three other co-locating QTL include seed size traits. One major QTL, *qMTOS-20*, was detected on chromosome 20 that co-locates for oil, seed length,

seed area, and TSW. This QTL explained 12.2% of oil (%) phenotypic variance, and 10.9% of seed length. Another QTL located on chromosome 5, *qMTSSPP-5*, co-locates for seed area, seed length, SPP, and TSW. Additionally, *qMTPS-18* on chromosome 18 co-locates for seed area, seed length, pod area, pod length, and TSW. In addition to this QTL, three additional significant QTL were detected that affected seed area, length, and/or width. In total, these QTL explain between 5.9% and 10.9% of the phenotypic variance for seed size, and in total account for 37.3% of the seed area phenotypic variance, 41.2% of seed length, and 6.2% of seed width. For all these QTL, except *qMTOS-20*, the additive effect was negative, meaning that it the positive mean impact comes from the Pry allele. Interestingly, only three QTL were located for TSW and all of them co-located with other traits. The total phenotypic variance explained 21.6%, much lower than seed size. In addition to the single co-locating QTL described above, seven additional QTL were located for pod size. These loci explained between 6% and 9.6% of the phenotypic variance for pod size, including 44.3% of total pod area, 23.7% of pod length, and 16.7% of pod width. Four of them have a negative additive effect, and four have a positive additive effect. Five QTL were detected for oil (%) explaining between 5.8% and 12.2% of phenotypic variance, and in total explain 43.2% of the total phenotypic variance. All of the QTL have positive additive effects. Besides the QTL described above, four more significant QTL were located for SPP, explaining a combined 39.9% of the phenotypic variance. Again, the additive effects are either positive or negative: three are positive, influenced by the Sun allele effect, and two are negative, influenced by the Pry allele.

QTL *qMTH-16*, was only found in the mean environments suggesting that peaks were close to significant in different environments, and when the four environments were averaged, to create the mean environment, a significant peak was located. This QTL has a high LOD score, explains 10.6% of the phenotypic variance seen in the population and has an additive effect of 4.81 cm.

Table 1. Trait mean for Suneson and Pryzeth.

Means of Suneson and Pryzeth					
Trait	**Suneson**	**±SD**	**Pryzeth**	**±SD**	**Difference**
Seed Area (mm^2)	1.35	0.04	2.24	0.12	0.89*
Seed Length (mm)	1.75	0.03	2.4	0.05	0.65*
Seed Width (mm)	0.996	0.03	1.17	0.04	0.18*
Pod Area (mm^2)	20.77	1.57	37.43	1.69	16.66*
Pod Length (mm)	7.98	0.51	9.97	0.61	1.99*
Pod Width (mm)	3.94	0.15	5.69	0.13	1.75*
SPP (#)	11.7	1.53	13.19	1.14	1.49*
TSW (grams)	1.09	0.06	1.86	0.15	0.77*
DTF	48.17	0.97	43.63	1.56	4.54*
DTM	93.72	1.98	87.63	1.43	6.09*
Height (cm)	78.99	6.32	73.02	4.05	5.97*
Oil (%)	37.15	1.37	33.69	0.87	3.46*

* = $P < .001$ attained from T-Test
SPP = seeds per pod
TSW = thousand seed weight
DTF = days to flowering
DTM = days to maturity

Figure 1. Phenotypic summary of raw data from the Pry/Sun bi-parental population. Histograms represent the data from the 190 RIL combined over both years and treatments (n=760). The red line in the histogram represents the mean of the population. The two box-plots under the histograms represent the combined data for the two parent varieties over both years and treatments (n=30). Sun = Red , Pry = Blue.

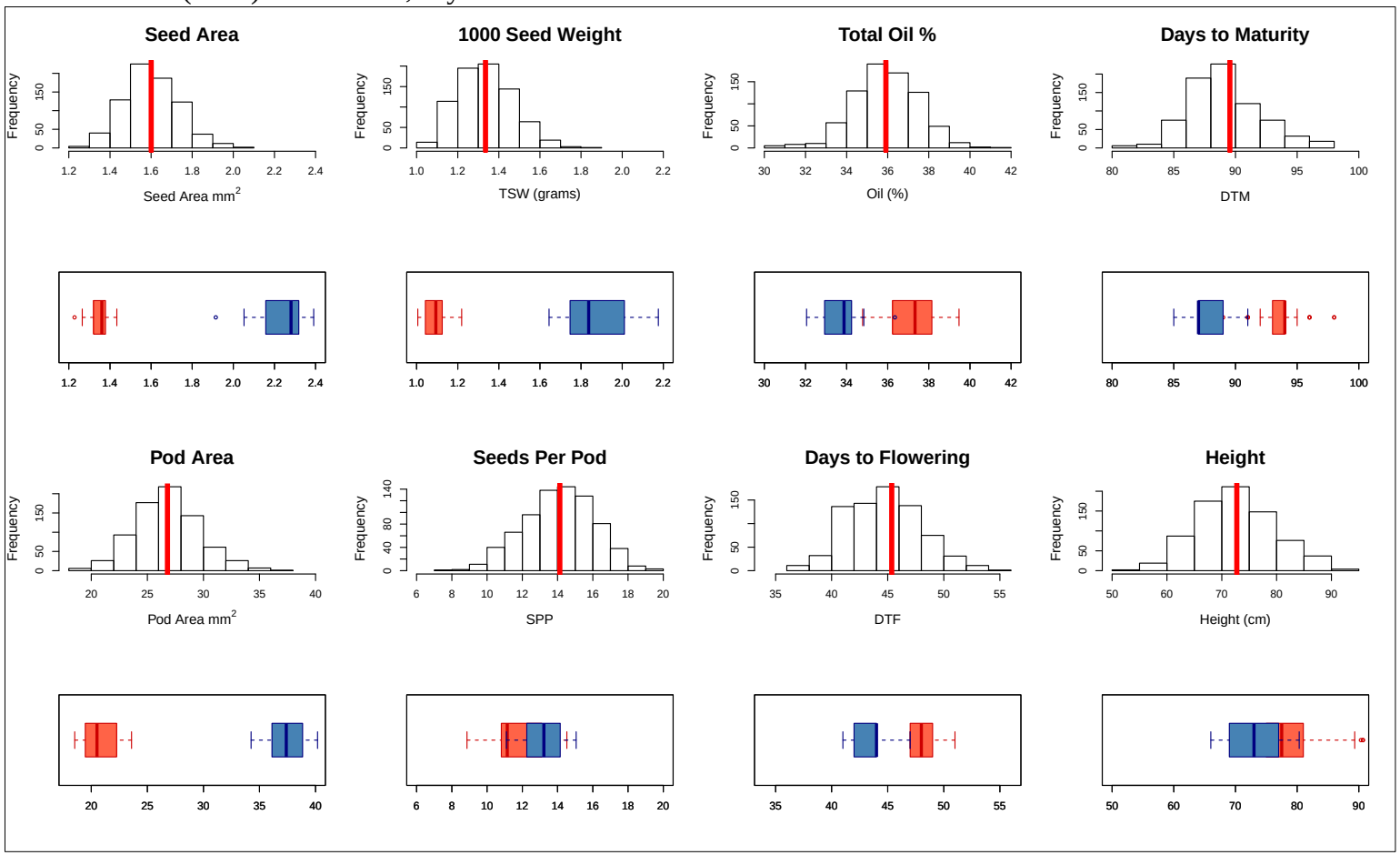


Table 2. EBLUP data for the RIL population.

Phenotypic Summary of EBLUP Data									
	2017 F_5		2018 F_6						
Trait	MEAN	±SE	MEAN	±SE	Range Over Both Years & Treatment	Year to Year Signifcance	Treatment Signifcance	H^2	±SE
Seed Area (mm2)	1.56	0.004	1.64	0.004	1.33 - 1.94	*	*	0.85	0.017
Seed Length (mm)	1.96	0.004	1.99	0.003	1.76 - 2.19	*	*	0.88	0.014
Seed Width (mm)	1.02	0.001	1.04	0.001	0.95 - 1.14	*	*	0.79	0.023
Pod Area (mm2)	26.41	0.105	27.2	0.109	21.10 - 35.66	*	*	0.80	0.022
Pod Length (mm)	8.14	0.024	8.54	0.02	6.51 - 9.77	*	*	0.83	0.019
Pod Width (mm)	4.68	0.01	4.72	0.012	4.00 - 5.41	*	*	0.80	0.022
SPP (#)	14.24	0.064	14.03	0.071	9.77 - 17.95	*		0.72	0.031
TSW (grams)	1.29	0.004	1.38	0.004	1.09 - 1.65	*	*	0.80	0.023
Oil (%)	35.85	0.064	35.97	0.071	30.83 - 40.74			0.66	0.036
DTF	44.94	0.117	45.77	0.155	38.72 - 53.85		*	0.70	0.034
DTM	89.55	0.121	89.54	0.104	81.69 - 96.08		*	0.58	0.043
Height (cm)	69.22	0.133	76.34	0.227	61.32 - 88.02	*	*	0.58	0.048

* = P < .001 attained from ANOVA
H^2 = broad-sense heritability
SPP = Seeds Per Pod
TSW = Thousand Seed Weight
DTF = Days to flowering
DTM = Days to Maturity

Table 3. 2017 and 2018 weather data summary.

Arthur H. Post Reasearch Farm Weather Data								
Month	2017 TMax	2017 TMin	2018 TMax	2018 TMin	2017 PCPN	2017 IRR	2018 PCPN	2018 IRR
May	19.16	3.87	20.16	6.38	6.50	0.00	7.39	0.00
June	24.19	7.68	22.19	8.31	5.66	5.08	9.14	2.54
July	30.86	12.71	27.76	10.46	0.28	7.62	0.51	8.89
August	28.35	10.43	27.09	9.52	1.40	0.00	3.15	0.00
Average[Total	25.64	8.67	24.30	8.67	13.84	12.70	20.19	11.43

TMax = Average maximum temperature (ºC)
TMin = Average minimum temperature (ºC)
PCPN = Precipitation (cm)
IRR = Addition irrigation (cm)

The irrigated treatment received just PCPN, the dryland treatment received PCPN + IRR

Table 4. Pearson correlation coefficients for all agronomic traits covering all years and treatments.

Correlation Matrix For All Agronomic Traits

	Seed Area	Seed Length	Seed Width	Pod Area	Pod Length	Pod Width	SPP	DTF	DTM	Height	Oil (%)	TSW
Seed Area	1											
Seed Length	0.92***	1										
Seed Width	0.88***	0.65***	1									
Pod Area	0.39***	0.43***	0.22***	1								
Pod Length	0.34***	0.39***	0.16***	0.84***	1							
Pod Width	0.35***	0.31***	0.30***	0.70***	0.31***	1						
SPP	-0.30***	-0.24***	-0.31***	0.39***	0.31***	0.25***	1					
DTF	-0.11	-0.21***	0.03	-0.07	-0.08	0.10	0.09	1				
DTM	-0.06	-0.14***	0.02	0.01	-0.07	0.11	0.12**	0.62***	1			
Height	0.31***	0.16***	0.34***	0.10	0.23***	0.12*	-0.18	0.42***	0.25***	1		
Oil (%)	-0.20***	-0.32***	-0.04	-0.14***	-0.16***	-0.07	0.28	0.07	0.08	0.03	1	
TSW	0.89***	0.79***	0.82***	0.35***	0.34***	0.30***	-0.32	-0.04	-0.02	0.35***	-0.18***	1

Significance Level: *,**,*** = P < 0.05, 0.01, and 0.001, respectively
SPP = seeds per pod
TSW = thousand seed weight
DTF = days to flowering
DTM = days to maturity

Figure 2. Trait complex between seed area, pod area, and SPP (seeds per pod).

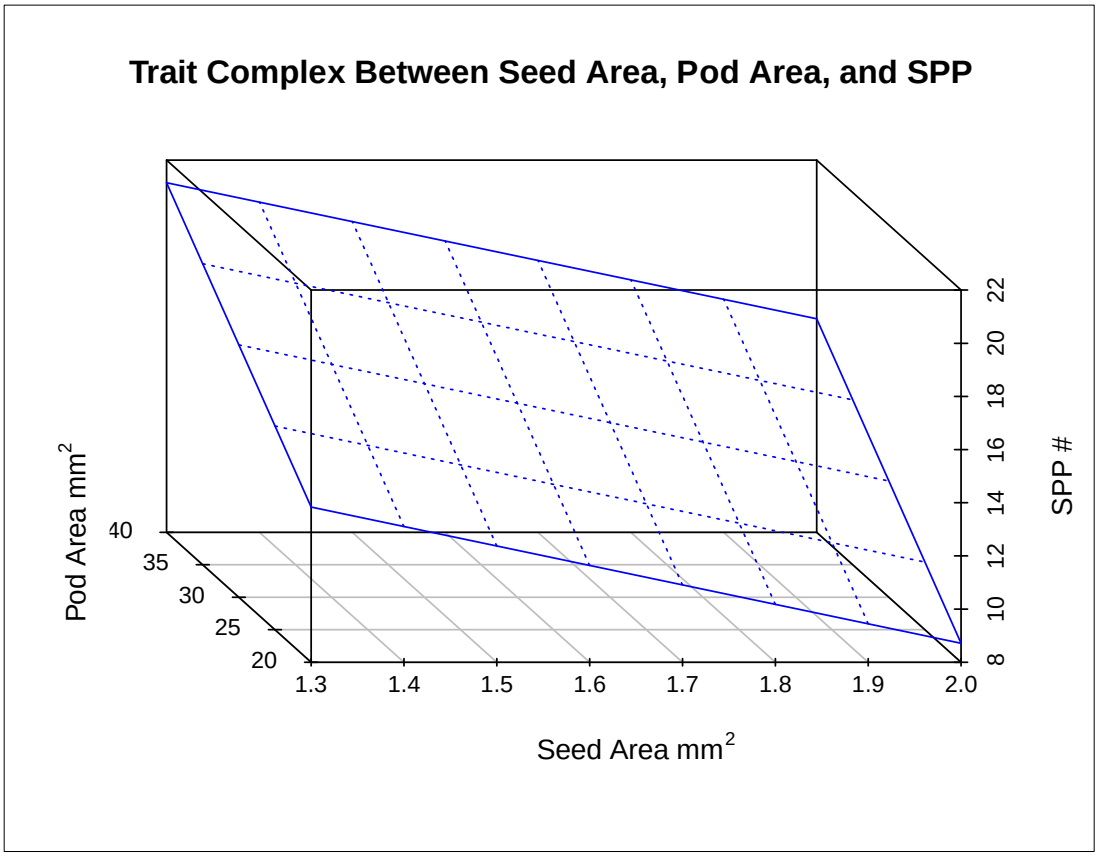
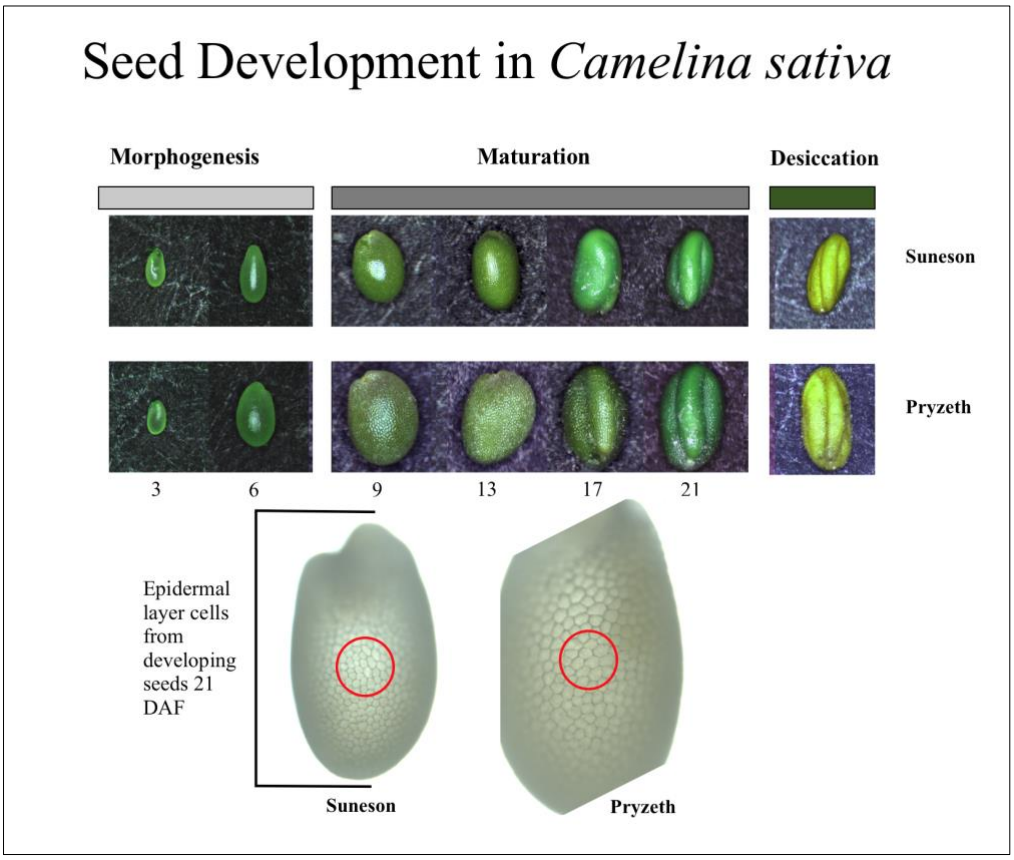


Figure 3. Seed development of the two parents of the bi-parental population. Plants were greenhouse grown, and numbers under developmental photos represent days after flowering (DAF) each photo set was taken.



Photos: Dr. Huang Li

Table 5. Linkage map summary.

Chromosome	Length (cM)	Number of Loci	length/marker (cM)
1	59.65	45	1.33
2	74.69	126	0.59
3	103.73	155	0.67
4	84.27	139	0.61
5	74.81	54	1.39
6	52.64	101	0.52
7	103.09	136	0.76
8	85.33	107	0.80
9	83.29	55	1.51
10	38.38	16	2.40
11	124.13	190	0.65
12	75.48	129	0.59
13	74.51	127	0.59
14	89.5	84	1.07
15	75.35	93	0.81
16	99.62	134	0.74
17	81.8	46	1.78
18	94.6	87	1.09
19	86.69	103	0.84
20	76.78	76	1.01
Total|Average	**1638.34**	**2003**	**0.99**

Table 6. RIL QTL summary for single traits.

Single Trait QTL Summary							
Trait	**Name-chromosome**	**Interval Markers**	**Position of the Peak (cM)**	**LOD**	**R² (%)**	**Add. Effect**	**Environment**
Height	*qMTH-16*	MC00008134 MC00918158	104	4.585	10.6	-4.807	5
	qMTH-15	MC01291292 MC01550755	89	3.067	7.2	-1.382	3,5
	qMTH-5	MC00435234 MC00400931	82	2.922	6.9	-0.929	2
Oil	*qMTO-4*	MC00271945 MC01035370	40	4.031	9.4	0.972	1,4,5
	qMTO-5	MC00000725 MC00361233	99	3.649	8.5	0.752	3
	qMTO-9	MC00333698 MC00091034	134	3.118	7.3	0.331	4
	qMTO-8	MC00002390 MC01502911	117	2.467	5.8	0.274	1,5
Pod Size							
PL	*qMTPL-7*	MC00104833 MC00018644	208	4.118	9.6	-0.185	3
PW	*qMTPW-13*	MC00214025 MC00915002	34	3.631	8.5	-0.079	1,3,5
PA	*qMTPAPW-6*	MC01139470	60	3.456	8.1	0.979	1,2,4,5
PW		MC01567971		3.514	8.2	0.114	1,2,4,5
PA	*qMTPA-18*	MC01513201 MC00897686	133	3.252	7.7	2.001	2
PA	*qMTPAPL-11*	MC00392212	46	3.171	7.4	-0.682	1,5
PL		MC00242947		3.257	7.6	-0.146	1,4,5
PA	*qMTPA-2*	MC00318186 MC00034959	130	2.648	6.2	0.792	1,4,5
PA	*qMTPA-9*	MC01137422 MC01596918	119	2.545	6.0	1.114	4,5
Seed Size							
SA	*qMTSASL-2*	MC00130382	23	3.435	8	0.041	1
SL		MC00703018		3.605	8.4	0.036	1
SA	*qMTSASL-8*	MC00613317	79	3.169	7.4	-0.048	4
SL		MC01098735		3.11	7.3	-0.035	4
SW	*qMTSW-8*	MC00154746 MC01364407	7	2.598	6.2	-0.005	2

SPP	*qMTSPP-11*	MC00114439 MC00899229	81	3.493	8.2	-0.372	1
	qMTSPP-13	MC01276923 MC00002954	116	3.433	8.0	1.221	3,5
	qMTSPP-19	MC00870165 MC00066298	36	3.264	7.7	1.922	3
	qMTSPP-2	MC00202412 MC00002278	138	2.981	7.0	-0.464	1,2,5

LOD = Log10 likelihood ratio
R^2 = The percent of total phenotypic variance explained
Add. Effect = Positive or negative effect of the Suneson allele
Environment = 1- Dry2017, 2 – Irr2017, 3 – Dry2018, 4 – Irr2018, 5 – Mean of all four environment
SPP = seeds per pod, TSW = thousand seed weight, DTF = days to flowering, DTM = days to maturity, SA = seed area, SL = seed length, SW = seed width, PA = pod area, PL = pod length, PW = pod width

Table 7. RIL QTL summary for co-located traits.

Co-located Trait QTL Summary							
Trait	**Name-chromsome**	**Interval Markers**	**Position of the Peak (cM)**	**LOD**	**R² (%)**	**Add. Effect**	**Environment**
DTF	*qMTDEV-12*	MC00040561	162	2.874	6.8	0.927	2,5
DTM		MC01579823		2.984	7.0	0.64	2,5
DTF	*qMTDEV-15*	MC01550755	88	2.941	6.9	-0.901	3
DTM		MC01291292		2.85	6.7	-0.667	3
DTF	*qMTDEV-7*	MC00456247	36	2.711	6.4	-0.936	4,5
DTM		MC00999024		3.503	8.2	-0.716	3,4,5
H				4.181	9.7	-1.447	3,4,5
SL	*qMTOS-20*	MC00141034	84	4.744	10.9	-0.047	ALL
SA		MC00470176		3.766	8.8	-0.05	ALL
O				5.32	12.2	0.882	ALL
TSW				2.725	6.4	-0.041	3,5
SL	*qMTSSPP-5*	MC00778086	62	2.581	6.1	-0.021	2,5
SA		MC00126966		2.508	5.9	-0.025	2,5
SPP				2.952	7.0	0.385	1
TSW				2.963	7.0	-0.033	2
SL	*qMTPS-18*	MC00977883	100	3.629	8.5	-0.049	1,3,4,5
SA		MC01327465		3.051	7.2	-0.054	1,5
PA				3.833	8.9	-1.848	2,3,4,5
PL				2.766	6.5	-0.338	1,3,4,5
TSW				3.495	8.2	-0.058	1

LOD = Log10 likelihood ratio
R^2 = The percent of total phenotypic variance explained
Add. Effect = Positive or negative effect of the Suneson allele
Environment = 1- Dry2017, 2 – Irr2017, 3 – Dry2018, 4 – Irr2018, 5 – Mean of all four environment
SPP = seeds per pod, TSW = thousand seed weight, DTF = days to flowering, DTM = days to maturity, SA = seed area, SL = seed length, SW = seed width, PA = pod area, PL = pod length, PW = pod width

Table 8. Summary of allelic frequencies for QTL in the Pry/Sun RIL F_5 population.

Allelic Friquencies for QTL					
Name	**Markers**	**n**	**Suneson (%)**	**Pryzeth (%)**	**H (%)**
qMTH-15	MC01291292	167	46.11	46.11	7.78
	MC01550755	183	46.45	48.09	5.46
qMTH-16	MC00008134	169	43.79	50.30	5.92
	MC00918158	159	55.97	40.88	3.14
qMTH-5	MC00435234	189	50.26	46.56	3.17
	MC00400931	177	51.41	46.33	2.26
qMTO-8	MC00002390	182	51.10	43.96	4.95
	MC01502911	185	54.05	43.24	2.70
qMTO-4	MC00271945	155	49.03	47.74	3.23
	MC01035370	187	44.39	50.80	4.81
qMTO-9	MC00333698	184	51.09	48.91	0.00
	MC00091034	187	47.06	49.20	3.74
qMTO-5	MC00000725	186	46.77	48.92	4.30
	MC00361233	180	42.78	47.22	10.00
qMTPAPW-6	MC01139470	186	48.39	48.39	3.23
	MC01567971	172	50.58	48.84	0.58
qMTPA-9	MC01137422	185	49.19	47.03	3.78
	MC01596918	187	48.13	47.06	4.81
qMTPW-13	MC00214025	187	43.32	55.61	1.07
	MC00915002	187	45.45	48.13	6.42
qMTPA-18	MC01513201	185	44.32	52.97	2.70
	MC00897686	153	52.29	46.41	1.31
qMTPAPL-11	MC00392212	185	45.95	48.11	5.95
	MC00242947	189	46.56	48.15	5.29
qMTPL-7	MC00104833	182	53.30	43.41	3.30
	MC00018644	186	52.69	44.09	3.23
qMTPA-2	MC00318186	180	45.00	42.22	12.78
	MC00034959	188	48.40	47.34	4.26
qMTSASL-8	MC00613317	189	48.15	48.68	3.17
	MC01098735	179	44.69	48.60	6.70
qMTSW-13	MC00154746	189	50.26	46.56	3.17
	MC01364407	185	51.89	44.86	3.24
qMTSASL-2	MC00130382	163	52.15	45.40	2.45
	MC00703018	184	46.74	46.20	7.07
qMTSPP-11	MC00114439	171	41.52	54.39	4.09
	MC00899229	186	43.01	50.00	6.99
qMTSPP-2	MC00202412	189	47.62	47.09	5.29
	MC00002278	170	46.47	42.94	10.59
qMTSPP-19	MC00870165	189	44.44	51.85	3.70
	MC00066298	173	45.66	52.02	2.31
qMTSPP-13	MC01276923	163	52.15	44.17	3.68
	MC00002954	155	50.32	46.45	3.23
qMTDEV-12	MC00040561	189	43.92	48.15	7.94
	MC01579823	184	43.48	52.17	4.35
qMTDEV-15	MC01550755	183	46.45	48.09	5.46
	MC01291292	167	46.11	46.11	7.78
qMTDEV-7	MC00456247	188	50.53	43.09	6.38
	MC00999024	182	50.00	46.15	3.85
qMTOSED-20	MC00141034	188	49.00	47.00	4.00
	MC00470176	189	50.79	44.44	4.76
qMTSSPP-5	MC00778086	169	48.52	47.34	4.14
	MC00126966	189	50.79	48.68	0.53
qMTPS-18	MC00977883	187	42.25	51.34	6.42
	MC01327465	187	44.92	55.08	0.00
Average			**47.88**	**47.65**	**4.47**

CHAPTER FIVE

DISCUSSION

Seed Size and Other Agronomic Traits in the RIL Population

For most traits measured of the RIL population, ANOVA results showed significant differences ($P < 0.001$) between years and between treatments. The year to year differences may partly be attributed to the use of two successive generation (F_5 in 2017 and F_6 in 2018). It is estimated that during the F_5 generation the genomes of the RIL population are ~6% heterozygous, and in the F_6 generation this is reduced to ~3%. The markers flanking QTL are, on average, 4.46% heterozygous for the F_5 generation. This reduction of heterozygosity in the population could result in a change in phenotypic traits. However, because of the high broad–sense heritability of most of the traits it was most likely environmental differences and cultural practices that influenced phenotypes. The two different planting densities could have had an effect on phenotypic results, but camelina is remarkably stable when subjected to highly reduced planting densities (McVay & Khan, 2011), seed size in particular can remain consistent over an extremely wide range of plant densities (Harper et al., 1970). Just visually, the space-planted plants (1 lb/acre) had much more branching than plants planted at higher density. This branching led to the plants "filling in" a lot of the space. This plasticity that camelina shows at different densities is one characteristic that allows its ability to outcompete weed and mature early. The year 2017 was remarkably dry with higher temperature started earlier than usual. The higher temperatures during flowering and pod formation

could have played a factor in phenotypes with all the averages for seed and pod characteristics, except for SPP, being smaller in 2017. Height had an average difference of 7.12 cm between the two years, and the most likely cause is environmental conditions.

Seed size (area, length, and width), pod size (area, length, and width), and TSW all show high broad–sense heritably, with H^2 values of around 0.80. Seed area and width have the highest H^2 (0.85 and 0.88, respectively). These findings are consistent with what have been reported for heritability of TSW in camelina 0.94 (Gehringer et al., 2006) and 0.87 (Enjalbert, 2012). In *Brassica napus* seed size/weight is also highly heritable with H^2 values being reported of 0.85 (Li et al., 2018), 0.91 (D. Cai et al., 2014), 0.82, 0.90 (Shi et al., 2009), and 0.83 (Fan et al., 2010). Interestingly, the calculated heritability in this study ($H^2 = 0.66$) is much lower than what has been shown in other camelina studies, 0.89 (Gehringer et al., 2006) and 0.87 (Enjalbert, 2012). High H^2 values were found for *Brassica napus* as well, 0.86 and 0.91 (F. Sun et al., 2016) 0.95 (Jiang et al., 2014). Here we report SPP to have a fairly high heritability of 0.72, and this is higher than what previously for camelina, 0.57 (Enjalbert, 2012). The additive effect for seed size QTL are all low ranging from -0.005 mm for seed length to -0.054 mm^2 for seed area. This may be an effect of the high broad–sense heritability of these traits; resulting in low variation.

This is the first study to show the broad-sense heritability of pod size in camelina. Pod area, length, width all showed high heritability (0.80, 0.83, and 0.80, respectively). High heritability, 0.91 (Li et al., 2014) and 0.964 (Yang et al., 2012), has also been shown for silique length in *Brassica napus*. Also, seven significant QTL accounting for as much as 44.3% of pod size were discovered. This is the most out of any traits studied. These findings could support further research into pod traits as they should

be receptive to genetic manipulation. There is currently not much research into camelina pod size and morphology.

Relationship Between Seed Size, Pod Size, and SPP

Three primary yield components for any oilseed *Brassica* species are number of pods per plant, SPP, and seed weight (Diepenbrock & Grosse, 1995). Of these three traits, the highest correlation with yield is pod number per plant, then SPP, and finally seed weight (Shi et al., 2015). These traits are not entirely independent of one another, and form a trait complex that if manipulated correctly could increase productivity and in turn increase seed yield.

There is an inherent trade-off between seed size and SPP (Alonso-Blanco et al., 1999; Harper et al., 1970). In general, if seed size is increased the number of SPP decreases. This relationship has been phenotypically explored in camelina. Based on a series of reciprocal crosses of four pure *Camelina* lines, Tedin (1925) observed that there is a developmental correlation between seed size and SPP, and that increasing seed size tends to reduce the SPP. This trend was also shown in this study, and can be seen on the forward pane of the three dimensional plot of Figure 2. Tedin (1925) provided that this relationship continues only if the genic complex for pod size is unaltered, and again this trend can also be observed in this study. When looking at the two-dimensions of the forward pane (Figure 2), pod area is constant, and as seed area increases SPP decreases. When the third dimension of pod area is added and becomes variable, SPP becomes much more variable, and a whole host of phenotypes are produced. This includes lines

with large seed, small pods, and low SPP; small seed, large pods, and high SPP, and many phenotypes in-between.

Tedin (1925) also theorized that pod size and seed size are controlled by independent series of genes, and furthermore, that the genetics controlling length, width, and thickness of pods are likewise independent of each other. Here, pod width and length are only weakly positively correlated ($r = 0.31$), and this correlation is much less than seed width and length ($r=0.66$). Pod area and seed area are weakly positively correlated ($r=0.39$), and there is a weak negative correlation between seed area and SPP ($r = -0.30$), but a positive correlation between pod area and SPP ($r = 0.39$). Also, interestingly only one QTL co-localized for pod size and seed size, and one for seed size and SPP. QTL studies in *Arabidopsis* show little overlap between seed number and seed weight (Gnan, Priest, & Kover, 2014). This relationship seems to be reversed in *Brassica napus* in which high correlations between TSW and pod length has been observed, and the correlations between TSW and silique length and TSW and SPP are both highly positive ($r = 0.549$ and .879, respectively) (Ivanovska et al., 2007; Li et al., 2018). Also, multiple QTL have been discovered that co-located for both seed weight and silique length (Li et al., 2014; Shi et al., 2009; Zhang et al., 2011).

The application of irrigation significantly impacted seed traits, and has shown to impact branch number, SPP, and TSW, and oil (%) in *Brassica napus* (Clarke & Simpson, 1978; Smith, Wright, & Woodroofe, 1988). In this study irrigation had a significant impact ($P < 0.001$) on all seed pod characteristics except oil (%) and SPP. The non-significant impact on SPP is interesting because there was a significant increase of both seed and pod size between the two treatments. This relationship goes against a

major hypothesis with respect to the relationship between seed size and seed number in crops (Sadras, 2007), which states that seed number in crops having high plasticity allowing plants to produce a fewer or greater number of seeds depending on the availability of resources. If this hypothesis was applied to this study then there should be a significant difference in SPP between the irrigated and dryland treatments, because, in theory, the irrigated environment should have more resource availability. This significant difference may have not been observed because irrigation impacted both pod size and seed size relatively equally, allowing seeds to increase in size without significantly impacting SPP. Interestingly, the average SPP in the population is higher than either parent, and many members of the population fall far outside that of the two parents. As stated before, there are many variations of phenotypes in the population for seed size, pod size, and SPP. This could indicate that the progeny contained a combination of independent genetic alleles for pod size and seed size from the two parents that allowed for large phenotypic differences and a wide range of SPP. The biggest seeds and highest SPP were seen in lines that had the largest pods, and this relationship could be exploited by breeders and scientists to increase yield.

The larger pod size may have more to do with increased seed size and SPP beyond just providing more space. The pod walls along with leaves, and green stems provide the photosynthetic material that seed filling is dependent on (Inanaga, Kumura, & Murata, 1979). After flowering, leaves start to quickly senesce, and pod wall becomes an important source of CO_2 fixation to continue to support fruit growth (Hua et al., 2012).

Because of the high heritability of these traits, weak phenotypic correlations between them, little overlap in genetics, and a large range of resulting phenotypes, this

study can provide some more evidence that seed area, pod area, and SPP are under different genetic controls, and possibly can be manipulated independently. Selecting lines that simultaneously have larger seeds and larger pods could provide a way to increase seed size without suffering too great a loss in SPP. These lines may be utilized in a breeding program with the ability to increase seed yield.

Relationship Between Seed Size and Oil (%)

In congruence with Gehringer et al. (2006), this study saw a negative correlation between oil (%) and seed area, length, and width and ($r = -0.20$, -0.32, and -0.04, respectively). This is interesting because the increase in seed size from Sun to Pry is, in part, from an increase of cell size. If the increase in seed size is not a result of increased oil % then may be from an increase in protein content or a thicker seed coat. Alonso-Blanco et al. (1999) reported that the seed size difference between *Arabidopsis* accessions was from growth of the endosperm and/or growth of the seed coat. Preliminary data from Washington State University shows that protein content in camelina can vary a lot between different accessions, and protein content can be significantly negatively correlated with oil (%). As one is increased then the other is decreased. Further investigation is needed to determine exactly how Pry is larger than Sun, and the chemical makeup of the seeds should be tested.

QTL and Potential Major Genes

This study located 27 significant QTL, 15 of which were significant in multiple environments. Including the mean of all 4 environments in the analysis can help lessen

the effect of environment on the analysis and show peaks that may not be significant in one environment alone. QTL are spread throughout the genome which is consistent previous studies (Enjalbert, 2012; Gehringer et al., 2006). This study located 6 seed size QTL. This is more than any other QTL study published for camelina, with Gehringer et al. (2006) reporting 2 QTL for TSW, Enjalbert (2012) also reporting 2 for TSW, and Ayala Diaz (2014) reporting 1 for TSW. Comparisons of these QTL is difficult because the previous studies used different linkage maps and were not able to assign linkage groups to chromosomes. However, because of the close genetic link between *Arabidopsis* and camelina some of the same genes that play a role in *Arabidopsis* seed size may also play a role in camelina seed size. Encouragingly, several of the original QTL located by Alonso-Blanco et al. (1999) in *Arabidopsis* have been subsequently confirmed by other studies (El-Lithy et al., 2004; R. H. Moore et al., 2017; Rowan et al., 2011), and there have been successful efforts to fine map several of these QTL to find the causal genes. Gnan et al. (2014) suggest that two good candidate genes for the QTL found on chromosome 1 are *AAP1,* an amino acid transporter, and *KLUH,* a key gene for maternal control of seed size. Auxin Response Factors 2 (*ARF2),* important in various auxin-mediated developmental processes, has been fine mapped to a QTL located on chromosome 5 (Schruff et al., 2006). *APETALA 2 (AP2),* important in ovule and seed coat development, has been fine mapped to a QTL located on Chromosome 4 (Ohto, Fischer, Goldberg, Nakamura, & Harada, 2005). Luo, Dennis, Berger, Peacock, and Chaudhury (2005) suggest that two genes *Iku2,* important in endosperm development, and *MINI3,* also important in endosperm development, are likely to correspond to two other QTL in *Arabidopsis*. *AAP1* is an important amino acid transporter located in the

embryo. The *aap1 Arabidopsis* mutant showed lower levels of storage protein, but no significant difference in fatty-acid content (Sanders et al., 2009). Compared to wild-type, it also showed a reduced seed weight by 10%. QTL *qMTSSPP-5* may correspond to a copy of *AAP1* that is located on chromosome 5 in camelina.

A major family of genes that are involved in seed size is Auxin Response Factors (*ARF*). These genes are transcription factors that mediate auxin response genes. It is thought that these genes mediate auxin signaling by activating or repressing gene transcription, and are negative growth regulators. In *Arabidopsis, ARF2* mutants generated seeds that were 46% heavier than the wild-type (Schruff et al., 2006). A copy on *ARF2* is located on chromosome 18 and may correspond with *qMTPS-18*.

Another major gene involved in seed mass is *APETALA 2 (AP2)*. This gene is the founding member of a large family of transcription factors that has been primarily studied in *Arabidopsis*. *AP2* mutant lines have shown an increase in average seed mass that ranged from 2 to 104% over the wild-type (Jofuku, Omidyar, Gee, & Okamuro, 2005). Also using *AP2* mutants Ohto et al. (2005) also found that an increase in seed size was due to enlargement of cell size. The increase in seed size in *AP2* mutants mainly happened during the maturation growth phase, similar to what is seen between Sun and Pry, and the increase is from both cell size and cell number.

In a study by Luo et al. (2005), Both *Iku2* and *MINI3* mutants had smaller seed phenotypes compared to wild-type, and this was due tosmaller embryos. However, the cell size in the embryo was not decreased so the decrease in embryo size must be a result of decreased embryo cell number. Further investigation on embryo development may

shed light on wheather embryo size plays a role in the size difference between Sun and Pry seeds.

Six QTL co-located for multiple traits. These QTL could be either pleotropic or tightly-linked for these traits. A very promising one of these QTL, *qMTOS-20*, is located on chromosome 20. This QTL co-locates for seed area, seed length, oil (%), and TSW. Interestingly, the additive effects are in opposite directions. The increasing effect for oil (%) is from the Sun allele and the increasing effects for seed area, seed length, and TSW is from the Pry Allele. This raises the possibility that the Pry allele is promoting seed size while simultaneously obstructing oil accumulation. Since the additive effect for seed length is only -0.047 mm and the additive effect for oil is 0.882%. If this causal gene/genes are knocked down in Pry then a possible significant increase in oil content could be seen with only a small penalty on seed size.

Five significant QTL were located for oil (%). The number of QTL and range of phenotypic variation are similar to what have been reported in previous studies (Enjalbert, 2012; Gehringer et al., 2006). In this study, the most significant QTL, *qMTO-4*, is present in multiple environments and explains close to 1% of oil content, thus is a good candidate for potential fine mapping for the causal gene.

Exploiting the QTL for DTF, DTM, and height could help breeders produce varieties that are better suited for modern agricultural techniques (Al-barzinjy et al., 2003). Previous camelina QTL studies have located a QTL explaining ~25% of the phenotypic variance seen in DTF (Ayala Diaz, 2014; Enjalbert, 2012). A QTL with this high of significance was not found in this study, and again it is hard to compare QTL

from this study to previous studies that used different linkage maps, and that were not able to assign linkage groups to chromosomes.

QTL from this study may be validated through preforming similar studies with different mapping populations and mapping techniques. This includes distinctive bi-parental populations and accession populations for genome-wide association studies. Logical next steps for the QTL located in this study are to increase marker density and perform fine mapping studies. Increasing marker density around important regions of the linkage map may more accurately locate QTL, and help narrow the number of candidate genes. Also, RNA-sequencing at different seed development stages could indicate differential expression of genes between parental lines. These data could then be overlaid on validated QTL to show potential candidate genes associated with seed development.

CHAPTER SIX

CONCLUSION

This is the first study to use the Pry/Sun population for linkage map creation, and to perform a QTL analysis. The assembled linkage groups were assigned to the 20 chromosome that make up the *Camelina sativa* genome. QTL analysis shows that the genetic variation of seed size is highly quantitative, but comparable to other members of *Brassicaceae*. This study is the beginning of a long process to understand the genetic and environmental causes behind this variation. To understand this variation, seed size cannot be looked at alone. It forms a complex relationship with other important traits that show the potential to be manipulated. Manipulation could create phenotypes that are more productive and appealing to producers. Camelina has been a part of human history for thousands of years and only now we are discovering its true potential.

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